Gracia Dayton. *City Snails.* Watercolor on paper, 13″ × 22″.

Since CLL remains incurable with standard therapies due to development of disease refractoriness, continued research is needed to develop improved treatment outcomes for patients with CLL.

Treatment of Relapsed or Refractory Chronic Lymphocytic Leukemia

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Background: *Recent advances in the treatment of chronic lymphocytic leukemia (CLL) have moved beyond the traditional use of alkylating agents and purine analogs into regimens combining these two chemotherapy classes with monoclonal antibodies.*

Methods: *This article reviews treatments options for patients with relapsed or refractory CLL.*

Results: *Several studies have investigated novel agents in treating patients with 17p deletion, TP53 mutation, and fludarabine-refractory CLL, as well as patients with suboptimal response to intense treatment. These investigational agents include rituximab, alemtuzumab, ofatumumab, bendamustine, high-dose methylprednisolone, lenalidomide, lumiliximab, cyclin-dependent kinase inhibitors, small modular immunopharmaceuticals, Bcl-2 inhibitors, and histone deacetylase inhibitors. While these newer drugs and combination therapies have shown promise as treatment options for CLL, additional studies are needed to determine the immunosuppression, toxicities, and infections associated with their use.*

Conclusions: *Despite improvement in initial overall response rates, most patients relapse and require further treatment. CLL remains incurable with standard therapies due to development of disease refractoriness. As such, novel approaches such as those noted above warrant continued research to improve outcomes for patients with CLL.*

Introduction

Although recent advances in first-line therapy of chronic lymphocytic leukemia (CLL) have prolonged the duration of first remission, patients eventually experience disease progression, with limited therapeutic options.[1,2] In general, patients with asymptomatic relapsed disease should be followed expectantly, and indications for reinitiation of treatment should follow the same guidelines of the National Cancer Institute (NCI) and the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) for these patients as for previously untreated patients.[3,4]

Treatment selection of relapsed disease depends on a variety of factors, including patient age, performance status, duration of response to initial therapy, type of prior therapy, disease-related manifestations, and genetic abnormalities within the CLL cells. In most cases, first-line therapy can be repeated if the duration of response has lasted more than 1 year. On the other hand, treatment decisions are more challenging in patients with therapy-refractory disease or with the 17p deletion and TP53 mutation. Fluorescence in situ hybridization (FISH) imaging should be performed in all patients to determine cytogenetic abnormalities before making treatment

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decisions since many patients with relapsed CLL often acquire high-risk chromosomal abnormalities such as del11(11q12) and del17(17p13). These abnormalities result in resistance to most conventional chemotherapies used in the treatment of CLL. Among patients with high-risk prognostic factors, a subgroup of very high-risk CLL patients can be identified, with median survival below 24 to 36 months at the treatment time point. Based on this definition, patients with 17p deletion (5% to 8%), TP53 mutation (4% to 5%), and fludarabine-refractory CLL (5%), as well as patients with suboptimal response to intense treatment, belong in this group.[5] Fludarabine-refractory disease is defined as CLL that does not respond to fludarabine or that recurs within 6 months of treatment with a fludarabine-containing regimen. In contrast to the encouraging clinical outcomes of patients with fludarabine-sensitive disease, the prognosis of patients with fludarabine-refractory CLL is poor, with a median survival of less than 1 year.[6,7] A single-institution report of 99 patients with CLL refractory to fludarabine and alemtuzumab showed an overall response (OR) rate of 23% to salvage therapy other than stem cell transplantation (SCT), with no complete responses (CRs) and an overall survival (OS) of 9 months.[7] Thus, new agents are needed for the treatment of these patients, particularly for those with high-risk cytogenetic abnormalities such as 17p deletion, for which allogeneic SCT remains the only therapy that offers a prolonged progression-free survival (PFS). This review focuses on treatments options for patients with relapsed or refractory CLL.

Outcomes With Salvage Chemotherapy

Resistance to purine analogs is emerging as a major challenge in the management of patients with CLL. Most of these patients have already been exposed to and have become refractory to alkylating agents. The response rate to fludarabine alone in relapsed CLL ranges from 45% to 65%, and it is even lower in patients refractory to initial therapy (20%).[8-13] In a retrospective review of 147 patients with CLL who were refractory to fludarabine or had a remission of less than 6 months in duration after a fludarabine-containing regimen, the OR rate was 22% to the first salvage regimen, with a median survival of 10 months.[6] The response rate was highest in the group receiving combination therapy with a purine analog and alkylating agent (37%; 16 of 43 patients). The response rates for alkylating agent-refractory, alkylating agent-naive, and alkylating agent-sensitive CLL were 18%, 22%, and 40%, respectively, with respective survival rates of 8 months, 10 months, and 14 months. As expected, survival was longer in responders than in nonresponders. Among patients treated with a second salvage regimen, the best response rates were noted in those who underwent SCT (6 of 9 patients responded) and in those who received alemtuzumab (4 of 6 patients responded).[6] In patients with fludarabine-refractory CLL, the response rate to the combination of fludarabine and cyclophosphamide therapy was 39% (3% CR).[14]

In a study including 88 patients with CLL, the response rate to the combination of fludarabine and mitoxantrone was 50% in those with fludarabine-sensitive relapsed CLL and 25% in those with fludarabine-refractory disease, but overall the combination of these agents did not show a significant advantage over fludarabine alone.[15] The combination of fludarabine, cyclophosphamide, and mitoxantrone (FCM) in 60 patients with relapsed or refractory CLL induced a response in 78% with a CR rate of 50%, including 17% achieving negative minimal residual disease (MRD), and a partial response (PR) rate of 28%. The median duration of response was 19 months.[16] Similar results were reported in a smaller study that included 29 patients with relapsed or refractory CLL who achieved a response rate of 78% (32% CR, 25% nodular PR, 21% PR), a median duration of response of 19 months, and a median survival of 42 months.[17]

Rituximab as a Single Agent

Rituximab, a chimeric murine/human monoclonal antibody directed against CD20, has revolutionized the approach to patients with various B-cell malignancies, including CLL, because of its activity, its non–cross-resistance with chemotherapy, and its favorable safety profile.[18] In a pivotal phase II study, 50% of patients affected by follicular lymphoma achieved at least a PR.[19] However, an OR rate of 13% (all PRs) was observed in patients with relapsed or refractory small lymphocytic lymphoma (SLL) or CLL. Similar modest results have been reported in other small trials in which rituximab was administered as a single agent, with poor OR rates ranging from 6% to 35% (PRs) in the treatment of relapsed CLL.[20-24] Single-agent rituximab has inferior clinical activity in CLL where it is less effective for clearing disease from the bone marrow and has a short response duration.[24]

Attempts to enhance the efficacy of rituximab have included increasing either the dose or the dose density of its administration, which has improved the benefit of rituximab in CLL but neither with success. A study of single-agent rituximab using a thrice-weekly dosing schedule in 33 previously treated or untreated patients reported a 45% OR rate, including 42% PRs and an overall median duration of response of 10 months.[25] Thirteen patients experienced transient hypoxemia, hypotension, or dyspnea, and 1 patient discontinued therapy because of infusion-related toxicity despite a schedule of stepped-up dosing. Moreover, the median time to progression (TTP) was only 6 months. A group from the MD Anderson Cancer Center escalated the rituximab dose from 350 mg/m^2 to 2,250 mg/m^2 in 40 previously treated CLL cases and in 10 other patients with mature B-cell leukemias with improved response.[26] The OR rate was 40% (36% in CLL, all PRs), and response was correlated with dose: 22% for patients treated at a dose of 500 mg/m^2 to 825

mg/m², 43% at a dose of 1,000 mg/m² to 1,500 mg/m², and 75% for those treated at the highest dose of 2,250 mg/m². Benefit was predominantly in the blood and nodal compartment, with response duration approaching that achieved in follicular lymphoma.

Chemoimmunotherapy

The combination of fludarabine, cyclophosphamide, and rituximab (FCR) was developed based on in vitro and in vivo data indicating complementary activity with the combination. In vitro, fludarabine inhibits repair of cyclophosphamide-induced DNA interstrand cross links.[27] Fludarabine combined with cyclophosphamide (FC) increased the remission rate and duration compared with single-agent fludarabine in previously treated patients with CLL.[14,28] Exposure of fludarabine and rituximab in the human B-cell non-Hodgkin lymphoma (NHL) cell line produced a synergistic effect. Fludarabine downmodulated the membrane expression of complement inhibitory proteins CD46, CD55, and CD59 on B-cell CLL cells without significantly altering CD20 levels, thereby potentially making these cells more susceptible to rituximab-induced complement-dependent cytotoxicity.[29] Furthermore, in vitro studies have shown that rituximab sensitizes NHL to chemotherapeutic drugs through downregulation of antiapoptotic IL-10 autocrine/paracrine loops and bcl-2, making CLL B cells potentially more vulnerable to fludarabine-induced apoptosis.[30]

The efficacy, toxicity, and tolerability of chemoimmunotherapy with FCR were evaluated in previously treated patients with CLL. In one study, 177 patients received fludarabine 25 mg/m² per day on days 2–4 of cycle 1 and on days 1–3 of cycles 2 to 6, cyclophosphamide 250 mg/m² per day on days 2–4 of cycle 1 and on days 1–3 of cycles 2 to 6, and rituximab 375 mg/m² on day 1 of cycle 1 and 500 mg/m² on day 1 of cycles 2–6.[31] Cycles were repeated every 4 weeks. Of the 82% of patients who had previously received fludarabine, 21% were fludarabine-refractory. Among the 177 patients, 3%, 47%, and 50% of patients had low-risk, intermediate-risk, and high-risk disease, respectively. The 6 intended courses of treatment were administered to only 46% of patients. The OR rate was 73% (25% CR, 16% nodular PR, and 32% PR). Twelve (32%) of 37 CRs tested achieved molecular remission in bone marrow. The median TTP for patients achieving a CR, nodular PR, and PR was 39, 33, and 15 months, respectively. The estimated median survival for all patients was 42 months. Moreover, the estimated median survival for CRs and nodular PRs had not been reached but was estimated to be ≥ 45 and 30 months, respectively. The estimated median survival was 39 months for PRs and 11 months for nonresponders. The most common toxicities were neutropenia (grade 3/4 in 81% of patients), thrombocytopenia (grade 3/4 in 34%), anemia (grade 3/4 in 24%), fever of unknown origin (31%), major infections (16%), and minor infections (18%).

The Rituximab in the Study of Relapsed Chronic Lymphocytic Leukemia (REACH) study was an international, randomized, phase III trial initiated to directly compare FCR with FC alone in 552 patients with previously treated CLL.[32] Patients on both arms of the study received fludarabine 25 mg/m² per day and cyclophosphamide 250 mg/m² per day for 3 days, repeated every 28 days for a total of 6 cycles. Patients were randomly assigned to receive rituximab 375 mg/m² on day 1 of the first cycle and 500 mg/m² on day 1 of subsequent cycles. Approximately 18% of patients on both arms had prior fludarabine exposure, 26% were alkylator-refractory, and 55% had alkylator-sensitive disease. After a median follow-up of 25 months, rituximab significantly improved median PFS (20.6 months for FC vs 30.6 months for FCR; hazard ratio = 0.65, $P < .001$), OR rate (58% for FC vs 70% for FCR; $P = .0034$), CR rate (13% for FC vs 24.3% for FCR; $P < .001$), median duration of response (27.7 months for FC vs 39.6 for FCR; $P = .025$), and time to new CLL treatment (34.3 months for FC vs not reached for FCR; $P = .0024$). Median OS was 52 months for the FC group but was not reached for the FCR group, although this was not statistically significant. Grade 3/4 adverse events were higher in the FCR group, with slightly more neutropenia and thrombocytopenia and a similar incidence of grade 3/4 infections to FC. Fatal adverse events were also slightly higher with FCR (14% vs 10% with FC). The results of this trial demonstrate that FCR is an active regimen for treatment of relapsed or refractory CLL. However, FCR is not without significant toxicity in this setting, and its use is most appropriate for fit patients in whom prolongation of PFS is a goal.

The introduction of three structurally similar purine analogs — fludarabine,[33,34] cladribine,[35,36] and pentostatin[37,38] — in the late 1980s and early 1990s vitalized the development of new treatments for patients with CLL. Of these agents, fludarabine has been the most widely studied, but pentostatin appears to be the least myelosuppressive.[39] Based on the encouraging results obtained with the combination of pentostatin and cyclophosphamide in heavily pretreated patients with CLL,[40] rituximab was added to this regimen. In this study, 46 patients with previously treated CLL or other low-grade B-cell malignancies received pentostatin 4 mg/m², cyclophosphamide 600 mg/m², and rituximab 375 mg/m² (PCR), administered in 6 cycles at 3-week intervals.[41] For CLL patients, the OR rate was 75% and the CR rate was 25%. A response was seen in 75% of the fludarabine-refractory patients. The median duration of response was 25 months, and median time to treatment failure was 40 months. The median OS was 44 months. Myelosuppression was the most common side effect, with grade 3/4 infections developing in 28% of patients. The PCR regimen may be used as an alternative to FCR in relapsed or refractory CLL. Moreover, the combination of rituximab, cladribine, and cyclophosphamide (RCC)

was administered to 28 patients with recurrent or refractory CLL. The regimen showed an OR rate of 78% in these heavily pretreated patients; however, these were mostly PRs, with a CR rate of only 7%. The median PFS of responders was 12 months.[42]

A randomized, two-stage, phase II trial of FCM vs FCM plus rituximab (FCM-R) was conducted in 52 patients with relapsed CLL, 26 in each arm.[43] Responses were assessed according to the 2008 IWCLL criteria. The OR rates were 58% in the FCM arm and 65% in the FCM-R arm, and the respective CR rates were 15% and 42%. The toxicity of both regimens was acceptable. The addition of rituximab to FCM improved the response rates in relapsed CLL, resulting in more complete remissions and without additional safety concerns. Efficacy and safety should be fully tested in a randomized phase III trial.

Based on preclinical data demonstrating synergistic cytotoxicity between cisplatin and the nucleoside analogs cytarabine (Ara-C)[44] and fludarabine,[45-47] the combination regimen of oxaliplatin, a third-generation platinum compound, with fludarabine, Ara-C, and rituximab (OFAR1) was administered to 50 patients with Richter syndrome or fludarabine-refractory CLL in a phase I/II clinical trial.[48] The regimen consisted of increasing doses of oxaliplatin (17.5, 20, or 25 mg/m^2 per day) on days 1 to 4 (phase I), fludarabine 30 mg/m^2 on days 2 to 3, Ara-C 1 g/m^2 on days 2 to 3, rituximab 375 mg/m^2 on day 3 of cycle 1 and on day 1 of subsequent cycles, and pegfilgrastim 6 mg on day 6, every 4 weeks for a maximum of 6 courses. A total of 92% of patients had previously received a fludarabine-based regimen, including FCR and FCR plus alemtuzumab. OR rates were 50% in patients with Richter syndrome, 33% in patients with fludarabine-refractory CLL, and 50% among 14 patients who were ≥ 70 years of age. Responses were also encouraging in patients with high-risk cytogenetics, including 35% of patients with 17p deletion and 29% of those with 11q deletion. The median response duration was 10 months. Toxicities were mainly hematologic, and prolonged myelosuppression was not observed. Moreover, the regimen was well tolerated in patients who were ≥ 70 years of age. To enhance the response rate with a decrease in myelosuppression that were observed with OFAR1, a separate phase I/II clinical trial of 102 patients with Richter syndrome or fludarabine refractory CLL was conducted in which the daily dose of oxaliplatin was increased from 25 to 30 mg, the daily dose of Ara-C was decreased from 1 g/m^2 to 0.5 g/m^2, and the fludarabine and Ara-C were given on days 2–3 (level 1), days 2–4 (level 2), or days 2–5 (level 3) every 4 weeks (OFAR2).[49] In patients with CLL, the OR rate was 55% with OFAR2 vs 33% with OFAR1, and the median survival was 21.4 months with OFAR2 vs 13.8 months with OFAR1. Although the number of patients was small, OFAR1 was associated with a trend toward superior clinical outcomes in patients with Richter syndrome compared to OFAR2, and OFAR2 was associated with a trend towards superior clinical outcomes compared to OFAR1 in patients with relapsed/refractory CLL. OFAR is highly active and could be considered an option in heavily pretreated patients with fludarabine-refractory CLL.

Alemtuzumab

Alemtuzumab, a humanized IgG1γ monoclonal antibody directed against CD52, has shown notable activity as monotherapy for CLL. Although its detailed mechanism of action is not completely clear, the binding of alemtuzumab to CD52 on target cells may cause cell death by three different mechanisms: complement-dependent cytotoxicity,[50] antibody-dependent cellular cytotoxicity (ADCC),[51] and apoptosis.[52] It has also been shown to induce caspase-independent cell death in human CLL cells through a lipid raft-dependent mechanism. Additionally, unlike rituximab, it has also been shown to induce cell death in vitro in CLL cells through a mechanism that is independent of p53 status and caspase activation.[53] This unique mechanism of action has made alemtuzumab a potential option for patients with the high-risk cytogenetic marker del(17p13.1/TP53) mutation.

Alemtuzumab was initially approved in 2001 under accelerated approval following the pivotal multicenter, international phase II trial (CAM211) of single-agent alemtuzumab in 93 patients with relapsed or refractory B-cell CLL who were previously exposed to alkylating agents and had failed fludarabine therapy.[54] In this trial, the alemtuzumab dose was increased gradually to a target 30 mg given intravenously 3 times per week for a maximum of 12 weeks. The OR rate was 33% (2% CR, 31% PR), and the median duration of response was 8.7 months. The median OS was 16 months (32 months for responders). In this group of patients with essentially no treatment options, serious infections occurred in 27%. Two studies, however, showed that the infection risk of salvage treatment other than alemtuzumab was even higher.[7] Among 147 fludarabine-refractory patients receiving salvage treatment, the risk of major infection with fludarabine-alkylator combinations and anthracycline-containing regimens were 26% and 47% per course, respectively.[6] In a smaller series of similar patients receiving a variety of salvage therapies, major infections occurred in 89% of patients, with a risk of hospital admission for infection estimated at 17% per month.[55] Against this background, it became evident that the safety profile of alemtuzumab was comparatively favorable.[7] The efficacy and safety of alemtuzumab led to its subsequent approval and widespread use in the treatment of fludarabine-refractory CLL.

According to the results of a phase II study conducted by the German CLL Study Group, the subcutaneous administration of alemtuzumab appears to be as efficient as the intravenous route and may result in a marked reduction of the "first-dose" flu-like reactions that

frequently occur after intravenous administration.[56] The study included 103 patients with fludarabine-refractory CLL who received alemtuzumab subcutaneously in a dose-escalation schedule up to 30 mg 3 times weekly for up to 12 weeks. The OR rate was 34% (4% CR, 30% PR). The median PFS was 7.7 months, and the median OS was 19.1 months. For those with 17p deletion, the OR rate was 39%, the median PFS was 5.8 months, and the median OS was 18.3 months. A higher response rate of 75% was reported in a smaller phase II trial of subcutaneous alemtuzumab using the full 30-mg dose without the traditional dose escalation period in weeks 1 and 2.[57] However, in this small study of 20 patients, only those with good performance status were recruited, and none of them had bulky lymphadenopathy (> 5 cm), which may account for the higher response rate observed in this trial.

More encouraging results were seen with lower doses of subcutaneous alemtuzumab (10 mg 3 times per week for 18 weeks) administered to 49 patients with pretreated CLL.[58] The OR rate was 53% (27% CR). Among patients with an unfavorable karyotype, 45% responded, including 60% of those with the 17p aberration. After a median follow-up of 25 months, the median overall time to disease progression was 8 months (responders 12 months, nonresponders 4 months), and the median OS was 30 months. Grade 4 neutropenia was observed in 17%, and cytomegalovirus (CMV) reactivation occurred in 24% of the patients, with no CMV disease. This study confirms that low-dose subcutaneous alemtuzumab is effective in poor prognosis CLL and has a particularly favorable toxicity profile. These results led to the initiation of the ongoing registration trial for subcutaneous alemtuzumab (NCT00328198, clinicaltrials.gov).

To assess the possibility of enhancing the therapeutic efficacy and eradicating MRD, alemtuzumab has been combined with fludarabine by several investigators. Following the promising results in a small study that included 6 patients who were refractory to both drugs when administered as single agents,[59] a larger phase II trial studying the combination was conducted and enrolled 36 patients with relapsed or refractory CLL.[60] After an initial dose escalation of alemtuzumab over 3 days, alemtuzumab 30 mg plus fludarabine 30 mg/m^2 were administered on 3 consecutive days. Treatment was repeated after 28 days for up to 6 cycles. The OR rate was 83% (30% CR), the median OS was 35.6 months, and the median TTP was 12.9 months. The median OS for those achieving a CR had not been reached, and the median TTP was calculated as 21.9 months. Grade 3/4 toxicity was mainly hematologic (neutropenia 26%, leukopenia 44%, and thrombocytopenia 30%) and infectious (5% grade 3/4). Two patients with progressive disease developed fungal pneumonias, and 1 died of *Escherichia coli* sepsis. Only 2 subclinical CMV reactivations occurred, despite 80% of patients being CMV IgG-positive at baseline. This study suggested that the fludarabine/alemtuzumab combination may improve outcome for patients with relapsed or refractory CLL. To validate these observations, a phase III multicenter, open-label, randomized study was conducted to compare the efficacy and safety of the combination vs fludarabine alone as second-line therapy for patients with relapsed or refractory CLL.[61] The study randomized 335 patients and demonstrated a significantly higher OR rate (84.8% vs 67.9%), CR rate (30.4% vs 16.4%), and median PFS of (29.6 months vs 20.7 months) with the combination therapy vs fludarabine alone. Adverse events included cytopenias and infections and were similar for both arms. Infections including CMV occurred in 47% of patients receiving the combination and in 35% of those receiving fludarabine alone, respectively.

Following the impressive results of the fludarabine/alemtuzumab combination, a multicenter phase II trial combining fludarabine, cyclophosphamide, and alemtuzumab (FCCam) was conducted.[62] Fludarabine 25 mg/m^2 and cyclophosphamide 200 mg/m^2, both administered intravenously, plus alemtuzumab 30 mg administered subcutaneously were given on days 1–3 and repeated on day 29 for up to 6 cycles to 56 patients with relapsed or genetic high-risk CLL. The respective OR and CR rates were 68% and 22%. Another phase II study including 43 patients with relapsed or refractory CLL was recently presented that evaluated oral fludarabine 40 mg/m^2 per day and oral cyclophosphamide 250 mg/m^2 per day immediately followed by alemtuzumab 10 mg given subcutaneously per day on days 1–3.[63] A 2-day escalation of alemtuzumab was administered prior to the first cycle, and the dose was increased from 10 mg to 20 mg after the first cohort of 10 treated patients. The combination was repeated on day 29 for up to 6 cycles. A total of 93% of patients had previously been treated with a fludarabine-containing regimen, and 19% had previously received alemtuzumab. Most patients had poor prognostic factors, including 54% of patients with del(11q) or del(17p), with 28% showing 17p as the sole aberration and 68% with unmutated IgVH. The OR rate was 67% (30% CR, including MRD negativity in 6 patients). After a median follow-up of 16.7 months, the median PFS was 24.4 months. Grade 3/4 neutropenia was observed in 66% of the administered courses, symptomatic CMV reactivation occurred in 8 cases, and no CMV disease was recorded. Twelve major infections occurred, including hepatitis B virus reactivation (1 patient), pneumonia (8 episodes), and sepsis (3 episodes). Although no head-to-head comparison has been reported in the relapsed or refractory setting, results from a phase III trial comparing FCR to FCCam in the upfront setting showed an unfavorable safety profile, with decreased clinical benefit for the FCCam combination.[64]

In an attempt to further improve the efficacy of FCR, investigators at the MD Anderson Cancer Center added

alemtuzumab to the combination (CFAR).[65] The regimen consisted of cyclophosphamide 250 mg/m^2 on days 3–5, fludarabine 25 mg/m^2 on days 3–5, alemtuzumab 30 mg given intravenously on days 1, 3, and 5, and rituximab 375 mg/m^2 to 500 mg/m^2 on day 2, every 28 days for up to a total of 6 courses.[65] The results of a long-term follow-up of 80 patients who received CFAR as salvage therapy were recently presented. The median number of prior treatments was 3 (ranging from 1 to 14), with 75% of patient having received prior fludarabine combined with an alkylating agent (including FCR 56%), and 39% of patients were refractory to fludarabine. Of 79 patients assessed for response, there were 21 CRs (27%), 3 nodular PRs (4%), and 29 PRs (37%), for an OR rate of 67%. The median OS and TTF were 16.6 and 10.6 months, respectively, for all patients. For those achieving a CR, the median OS (50+ months) and TTF (28+ months) had not been reached. Grade 3/4 hematologic toxicity included neutropenia (62% of courses), thrombocytopenia (34%), and anemia (16%). Grade 3/4 infectious complications included pneumonia (n = 7) and sepsis (n = 3). Other effects included minor infections (n = 32), CMV reactivation (n = 13), and other herpes virus infections (n = 5). Although this study demonstrated feasibility and included heavily pretreated patients, whether CFAR provides any additional benefit over FCR in the relapsed or refractory setting remains unclear.

The combination of alemtuzumab with rituximab has also been evaluated by several phase I/II trials in relapsed or refractory CLL. The OR rate has ranged from 8% to 61%, with a CR rate ranging from 0% to 18%.[66-69] Overall, the combination was active with an acceptable safety profile in these small trials, and further exploration is recommended. Another promising approach is the combination of high-dose methylprednisolone (HDMP) with alemtuzumab, especially for patients with p53 defects. A phase II trial studied a regimen of up to 4 28-day cycles, each cycle consisting of intravenous methylprednisolone 1 g/m^2 on days 1–5 and alemtuzumab 30 mg 3 times weekly on days 1–28 after an initial week of dose escalation.[70] Alemtuzumab was given intravenously during cycle 1 and subcutaneously thereafter. Preliminary results on the 5 patients (3 previously treated) with clinically aggressive CLL with bulky lymphadenopathy showed that all 5 patients responded to therapy. Three patients achieved a CR, 1 a nodular PR, and 1 a PR. Toxicity was significant but appeared to be manageable with appropriate antimicrobial prophylaxis and surveillance. This regimen will be further evaluated within the NCRI UKCLL06 trial.

In summary, alemtuzumab monotherapy has resulted in OR rates ranging from 33% to 53% and a median duration of response of 8.7 to 15.4 months in patients with relapsed or refractory CLL.[54,56,71-73] The drug has effectively eradicated disease from the blood, bone marrow, and spleen, but it has been relatively ineffective at treating bulky lymphadenopathy, specifically if greater than 5 cm.[54,74] The reason for this differential response is not clear, although it has been postulated to be related to poor bioavailability of the drug in bulky sites, thus causing a low saturation of the binding sites on the surface of the neoplastic cells. Moreover, there may be variability in the immune effector mechanisms in lymph nodes compared with other sites.[75] Alemtuzumab has also demonstrated notable efficacy in treating CLL with 17p deletions or TP53 mutations, possibly due to the fact that it is not dependent on p53 for its antitumor activity.[73,76] Furthermore, a recent retrospective study confirmed the efficacy of alemtuzumab monotherapy in 105 pretreated (otherwise unselected) patients with CLL. The OR rate was 49% (all PRs) for 35 patients with 17p deletion. Interestingly, in this 17p deletion subgroup, the OR rate was only 29% if less than a 500-mg cumulative dose was given, and it was 62% if more than a 500-mg cumulative dose was given. Alemtuzumab has been associated with significant infectious complications, including opportunistic infections with *Pneumocystis jirovecii* and CMV. Though investigators are testing alemtuzumab both in combination with rituximab and other therapies and as consolidation to eradicate MRD, at this time alemtuzumab should not be used in either manner outside of a well-designed clinical trial.

Ofatumumab

Ofatumumab, newly approved by the US Food and Drug Administration (FDA), is an IgG1κ, fully humanized CD20 monoclonal antibody that targets an epitope distinct from the epitope recognized by rituximab. Its ADCC is similar to that of rituximab. However, ofatumumab delivers stronger complement-dependent cytotoxicity in in vitro models, even in malignant B cells with low CD20 expression levels,[77,78] and it has shown a slower off-rate and more stable CD20 binding.[77] Laboratory studies have shown ofatumumab to be effective at inducing lysis of several B-cell lines as well as killing fresh B-CLL cells resistant to rituximab.[77-79]

A phase I/II dose-escalating study evaluating ofatumumab in patients with relapsed and refractory CLL was initiated at 12 sites in the United States and Europe. This study evaluated 33 patients in 3 different dosing cohorts.[80] The majority of patients (27 of 33) received a total of 4 doses administered once weekly. The response rate for the cohort receiving an initial dose of 500 mg followed by three 2,000-mg doses was 50%, with partial remission observed in 13 of 26 patients. The drug was well tolerated, with the majority of adverse events being related to infusion reactions and grade 1/2 infections. Another single-arm phase II study currently recruiting patients is investigating the effects of ofatumumab retreatment and maintenance in CLL patients who received the monoclonal antibody in a previous study (Hx-CD20-406). Patients will receive 8 weekly infusions (300 mg, then 7 × 2,000-

mg doses), followed by 2,000-mg doses once monthly for 2 years. The study is expected to enroll 25 patients, and primary outcome measures should be reported after January 2012 (NCT00802737, clinicaltrials.gov).

Another larger study was conducted to evaluate patients with CLL refractory to alemtuzumab and fludarabine.[81] The interim analysis of this pivotal international study evaluated 138 patients, with 59 who were refractory to fludarabine and alemtuzumab (FA-ref) and 79 who were refractory to fludarabine but did not receive treatment with alemtuzumab due to bulky disease (BF-ref). Ofatumumab was administered as 8 weekly infusions followed by 4 monthly infusions. The first infusion was given at a dose of 300 mg, and the subsequent infusions were given at a dose of 2,000 mg. The OR rates were 58% and 47% in the FA-ref and BF-ref groups, respectively. All of these were PRs, with the exception of 1 CR. Complete resolution of constitutional symptoms and improved performance status occurred in 57% of patients in the FA-ref arm and in 48% of patients the BF-ref arm. The response was also significant in those who received prior rituximab therapy (OR rate 54% in the FA-ref arm and 44% in the BF-ref arm) as well as those who received prior treatment with the combination of fludarabine, cyclophosphamide, and rituximab (OR rates of 50% and 44% in the FA-ref and BF-ref groups, respectively). Furthermore, ofatumumab was also active in those with 17p deletion, with a response rate of 41% in the FA-ref group, although the response rate was 14% in the BF-ref group with 17p deletion. The median time to response was 1.8 months, and the median duration of response was 7.1 months in the FA-ref group and 5.6 months in the BF-ref group. The median PFS and OS survival times were 5.7 and 13.7 months in the FA-ref group, respectively, and 5.9 and 15.4 months in the BF-ref group. The most common adverse events occurring during treatment were infections (67%), infusion-related reactions (64% of patients in the FA-ref group and 61% of patients in the BF-ref group), nearly all of which were grade 1 or 2. These reactions predominantly occurred during the first and second infusions and subsided during the course of treatment: cough (18%), diarrhea (16%), anemia (16%), fatigue (15%), fever (15%), neutropenia (15%), dyspnea (13%), nausea (11%), and rash (10%). This study led to accelerated approval of ofatumumab in October 2009 by the FDA for patients with CLL refractory to fludarabine and alemtuzumab. The final results for the primary endpoint of this study in 206 enrolled patients were updated at the 2010 annual meeting of the American Society of Hematology.[82] The OR rate by an independent endpoint review committee evaluation was 51% for the FA-ref group and 44% for the BF-ref group. Two patients in the BF-ref group achieved a CR. The median duration of response was 5.7 months in the FA-ref group and 6 months in the BF-ref group. The median PFS was 5.5 months in both groups; the median OS was 14.2 months for the FA-ref group and 17.4 months for the BF-ref group.

Bendamustine

Bendamustine is a unique alkylating agent that was first synthesized in the early 1960s in the former East German Democratic Republic. Its structure has similarities to both alkylating agents and antimetabolites.[83] However, bendamustine displays a distinct pattern of activity unrelated to other DNA-alkylating agents. Its mechanisms of action include activation of DNA-damage stress response, inhibition of mitotic checkpoints, and induction of mitotic catastrophe. It induces many proapoptotic genes, thereby restoring p53/tumor suppressor gene function and causing a strong activation of intrinsic apoptosis. In addition, unlike other alkylators, bendamustine activates a base excision DNA repair pathway rather than an alkyltransferase DNA repair mechanism.[83] It also causes DNA double-strand breaks, but these are more extensive and durable than those produced by other alkylating agents. Moreover, bendamustine showed incomplete cross-resistance with other alkylating agents such as cyclophosphamide due to a substance-specific interaction between bendamustine and DNA.[84] For more than 30 years, bendamustine was used in East Germany to treat patients with NHL, CLL, multiple myeloma, Hodgkin lymphoma, and carcinoma of the breast. Following the German reunification, many new studies were initiated to better define the value of this drug in treating these conditions.[85]

Several small studies using different dosing schedules have demonstrated the clinical activity of bendamustine as a single agent in the treatment of relapsed CLL or alkylating agent-refractory CLL.[86-90] These studies have reported an OR rate between 56% and 93% and a CR rate between 7% and 29%. While doses of 100 mg/m^2 for monotherapy and 90 mg/m^2 in combination with rituximab are used to treat newly diagnosed CLL, dose-escalation trials in the relapsed or refractory setting suggest that these doses cause significant myelosuppression.[86-90] Therefore, a lower dose of 70 mg/m^2 on days 1 and 2 every 4 weeks should be used in pretreated patients, especially in those who have received fludarabine.[88] Bendamustine appears to be a good choice for second-line therapy due to its efficacy in pretreated CLL patients and its lack of significant cross-resistance with other alkylating agents or fludarabine.

Based on preclinical data showing that the addition of rituximab in vitro reduced the dose of bendamustine required to induce apoptosis in ex vivo B-CLL cells,[91] the combination of these two agents was studied.[92-94] The German CLL Study Group conducted a multicenter phase II trial of bendamustine in combination with rituximab in patients with relapsed CLL.[92] The study enrolled 81 patients who received bendamustine 70 mg/m^2 on days 1 and 2 and rituximab 375 mg/m^2 for cycle 1, then 500 mg/m^2 for subsequent cycles. Cycles were given every 28 days for up to 6 courses. In the 62 patients who were included in the response assessment, the OR rate was

77.4% and the CR rate was 14.5%. The OR rate was 92.3% for the subset with del(11q), 100% for trisomy 12, 44.4% for del(17p), and 74.4% for unmutated IgVH, suggesting that bendamustine may be active in high-risk relapsed CLL. Major toxicities included myelosuppression and infections. Grade 3/4 neutropenia was seen in 12%, and treatment-related mortality was reported at 3.7%.

Bendamustine has also been investigated in combination with other agents. A phase I/II study of bendamustine in escalating doses of 80 mg/m^2 to 240 mg/m^2 per cycle divided over 2 to 3 days, combined with mitoxantrone on day 1 in doses ranging from 8 mg/m^2 to 10 mg/m^2, was conducted in 22 patients with advanced CLL.[95] Treatment was repeated every 29 days for up to 6 cycles. The OR rate was 86% (27% CR). The median TTP was 10 months and median OS was 39 months. The recommended dose for further studies was bendamustine 150 mg/m^2 combined with mitoxantrone 10 mg/m^2. At the recommended doses, 7 of the 8 patients had grade 3/4 neutropenia, with 2 developing infections.

In a pilot single-institution phase I/II study, bendamustine was combined with mitoxantrone and rituximab in 54 patients with B-cell malignancies, including 21 with CLL.[96] Bendamustine (BM) was given at 80 mg/m^2 for 2 days, mitoxantrone (M) 10 mg/m^2 on day 1, and rituximab 375 mg/m^2 on days 8, 15, 22, and 29. Cycle 2 was started on day 36 and subsequent cycles of bendamustine were administered every 28 days. Treatment was discontinued once a CR or PR was achieved. Seventy-two percent of patients received only 2 cycles of therapy as they achieved a response. CLL patients had an OR rate of 95% (23% CR), and median TTP was 17 months. Toxicity was mainly hematologic in 43% of the patients, mostly grade 3/4 leukopenia and thrombocytopenia. Similar results were recently reported in a second study by the same group, using bendamustine combined with mitoxantrone and rituximab in 39 patients with relapsed or refractory CLL.[97] In the absence of larger studies, this regimen should not be considered at this time outside of clinical trials.

Preliminary results of a phase II trial of bendamustine combined with alemtuzumab in the relapsed-refractory setting were recently reported.[98] The protocol combined 4 cycles of bendamustine (70 mg/m^2 given intravenously on days 1 and 2, every 4 weeks) with continuous alemtuzumab (30 mg administered subcutaneously 3 times per week for 16 weeks). All patients had at least one high-risk feature from CD38, FISH, or IgVH mutation status. Results available from 7 patients showed 3 CRs, 2 PRs, and 1 stable disease for an OR rate of 71%. Toxicities were mostly hematologic and infectious, but the regimen was considered feasible and the study currently continues to recruit patients.

Despite extensive use with bendamustine for more than 40 years, a number of questions remain unanswered, such as the optimal dose and schedule. Moreover, bendamustine has some benefit over other agents used in CLL. For instance, bendamustine does not seem to be associated with autoimmune phenomenon, while fludarabine does appear to have an association. In fact, resolution of autoimmune phenomenon has been reported with bendamustine use in 2 patients, while no further episodes occurred in 7 patients with a prior history of autoimmune disorder.[86] Bendamustine seems to be a reasonable alternative for patients not suitable for fludarabine-based therapies due to either infectious risk or autoimmune phenomenon. Bendamustine also provides a reasonable option for patients not considered appropriate candidates for alemtuzumab because of recurrent infections or bulky lymphadenopathy. In addition, while clinicians tend to consider chlorambucil as initial therapy in elderly patients, the superiority of bendamustine over chlorambucil with no significant difference in the rate of grade 3/4 infections provides further rationale for its use.[85]

High-Dose Methylprednisolone

A number of prior studies suggest that high-dose corticosteroids are an active treatment in patients with relapsed/refractory CLL, including those with unfavorable cytogenetic characteristics.[99-101] Glucocorticoids are of interest in the treatment of high-risk CLL because they can kill lymphoid cells by a p53-independent mechanism and appear to be active in patients with 17p13.1 deletions.[101] Glucocorticoids also effectively reduce bulky lymphadenopathy and cause less myelosuppression than alkylating agents and purine nucleosides produce.[100] These characteristics make it possible to administer high-dose corticosteroid therapy to patients with cytopenias and to those in whom myelosuppression should be avoided.[102] Results of a phase II study combining rituximab, an anti-CD20 monoclonal antibody, with HDMP as a salvage regimen for the treatment of patients with fludarabine-refractory CLL showed an OR rate of 93%, with a CR rate of 36%.[103] Fourteen patients were treated with 3 cycles of rituximab 375 mg/m^2 weekly for 4 weeks in combination with HDMP 1 g/m^2 daily for 5 days. All patients were refractory to fludarabine, and 86% had high-risk disease according to the modified Rai classification. The median TTP was 15 months, and the median time to next treatment was 22 months. Median survival had not been reached after a median follow-up of 40 months. The regimen was well tolerated, and serious adverse events were rare. All patients were able to complete the scheduled treatment without delays or dosage reductions.[103] The CR and OR rates reported with the HDMP/rituximab combination are higher than those reported for each agent alone in patients with relapsed/refractory disease[25,26,100,101] and higher than those reported for FC, FCR, or FCM.[14,16,31] In addition, the response rates observed for the HDMP/rituximab combination are higher than those noted for alemtu-

zumab in patients with fludarabine-refractory disease, for which OR rates are approximately 30% and CRs are uncommon.[54,56] Another recent report from the same group showed a similar efficacy in the front-line setting: a 96% OR rate, with a CR rate of 32% using a reduced number of days of corticosteroids.[104] Although a larger single-institution review of 37 patients who were treated with the same HDMP/rituximab combination reported a lower OR rate of 78% (22% CR), a high response rate was seen for those with high-risk cytogenetic abnormalities, including OR rates of 55.6% and 66.7% for those with del(17p) and del(11q), respectively.[102] The HDMP/rituximab combination is an active regimen in patients with relapsed, refractory, and cytogenetically high-risk CLL. Further evaluation of this regimen in controlled trials appears warranted.

A study of the combination of HDMP and ofatumumab was recently reported in patients with CLL who were not considered good candidates for chemotherapy due to comorbidities, poor performance status, or profound cytopenias or due to refractory status to fludarabine and/or alemtuzumab.[105] Eight patients with progressive, symptomatic CLL were treated with HDMP 1 g/m^2 given intravenously daily × 3 every 28 days for 3 consecutive cycles and ofatumumab 300 mg as the initial dose, followed by 11 doses of 2,000 mg over a 6-month period. The median age was 69 years, and the median number of prior treatments was 4.5, including 4 patients who had previously received HDMP and rituximab and 2 patients who underwent matched unrelated-donor SCT. All patients had been previously treated with rituximab, 75% of the patients failed or were intolerant to fludarabine and/or alemtuzumab, and 75% had high-risk prognostic markers including unfavorable cytogenetics, unmutated IgVH region genes, or high expression levels of ZAP70. Most of the patients had bulky disease and splenomegaly. All patients completed the planned therapy with no major side effects or toxicities. No evidence of marrow suppression was seen, and even patients with pancytopenia improved their peripheral blood counts with this salvage regimen. Among the 8 patients, the OR rate was 50% (4 PRs), 2 patients had stable disease, and the remainder showed progressive disease. These data suggest that the combination of HDMP and **ofatumumab** is a safe and effective salvage regimen for high-risk CLL patients who otherwise are not candidates for additional treatment. Additional clinical studies of this combination are warranted.

Emerging Therapies and Investigational Agents

Lenalidomide

The immunomodulatory agent lenalidomide has demonstrated activity in CLL, and a discussion of this drug is included elsewhere in this issue (Carbadillo E, Veliz M, Komrokji R, et al; pp 54-67).

Lumiliximab

The success of the monoclonal antibodies rituximab and alemtuzumab in CLL suggests that cell surface antigens are promising targets for the treatment of CLL. CLL cells exhibit high expression of CD23, while non-CLL cells only minimally express this antigen. As such, CD23 has become a potential target for directed therapy. Lumiliximab is a macaque-human primatized monoclonal antibody that targets the CD23 antigen.[106] In a phase I trial, investigators found that lumiliximab was well tolerated, but its efficacy was limited to reduction of lymphadenopathy and lymphocytosis, with no documented CRs or PRs.[106] Since preclinical data suggested an enhancement of the antitumor effect when lumiliximab was combined with rituximab or fludarabine, investigators tested the safety and efficacy of the combination of FCR with lumiliximab for treatment of relapsed CLL.[107] In this phase I/II trial, the toxicity of the combination appeared no different from that which was previously reported with FCR in treatment of relapsed CLL. FCR plus lumiliximab resulted in an OR rate of 65% and a CR rate of 52%. Results from a phase III study comparing FCR to FCR plus lumiliximab in relapsed CLL did not confirm a benefit in terms of improved response rate of PFS with the addition of lumiliximab to FCR.[75]

GA101

GA101 is the first humanized and glycoengineered type II monoclonal anti-CD20 antibody to enter clinical trials. It binds CD20 in a different orientation than rituximab and over a larger surface area.[108] In preclinical in vivo lymphoma models, GA101 has shown superior efficacy[109] as well as increased ADCC and strongly enhanced direct cell death compared with rituximab[110] and ofatumumab.[111] A phase I study of GA101 was initiated in 13 patients with relapsed or refractory disease.[110] The OR rate was 62% (1 CRi, 7 PRs, and 5 stable disease) with no clear dose relationship established. The drug was relatively well tolerated, with the most common grade 3/4 toxicity being transient neutropenia in 9 patients. Additional phase II/III studies are underway with this antibody as a single agent and in various combinations. Other newer anti-CD20 monoclonal antibodies such as veltuzumab, AME-133v, and TRU-015 are being investigated in early-phase clinical trials in patients with NHL or CLL.

Cyclin-Dependent Kinase Inhibitors

Flavopiridol

Flavopiridol (Alvocidib), a broad cyclin-dependent kinase (CDK) inhibitor, induces apoptosis of CLL cells in vitro[112] and is not dependent on p53 for its activity.[113] In initial phase I studies using a continuous-infusion dosing schedule in a variety of malignancies, no clinical activity was observed.[114,115] Detailed pharmacokinetic modeling led to the development of a novel dosing schedule designed to achieve target drug concentrations in

vivo.[116] Following initial phase I studies showing that a 30-minute intravenous bolus followed by 4-hour continuous intravenous infusion schedule was clinically active with a dose-limiting toxicity (DLT) of acute tumor lysis syndrome requiring hemodialysis,[114,115] a phase II trial was conducted to evaluate flavopiridol as a single agent for the treatment of 64 patients with relapsed CLL. The OR rate was 53% (1.6% CR, 5% nodular PR, and 47% PR).[117] Patients with genetically high-risk disease, including patients with del(17p) and del(11q), also had significant OR rates (57% and 50%, respectively). Patients with bulky lymphadenopathy had an OR rate of 51%. With the implementation of a standardized protocol to prevent severe tumor lysis syndrome, flavopiridol was administered safely. Adverse effects included cytopenias, diarrhea, nausea and vomiting, transaminitis, acute tumor lysis syndrome, and cytokine release syndrome (fever, flushing tachycardia, nausea). The interim results of a multicenter, international phase II clinical trial of flavopiridol among patients with fludarabine-refractory CLL or prolymphocytic leukemia arising from CLL was recently reported.[118] Patients received a bolus dose of 30 mg/m^2 given over 30 minutes followed by a continuous infusion of 30 mg/m^2 over 4 hours. If no tumor lysis syndrome was observed with the first dose, subsequent doses were administered with a continuous infusion of 50 mg/m^2 over 4 hours. Flavopiridol was given once weekly for 4 weeks, followed by a 2-week break, for up to 6 cycles (24 total doses). Of the planned 165 patients, 68 patients completed at least 2 cycles of therapy and were evaluated for efficacy and safety. A significant number of patients had high-risk cytogenetics, including 30% with del(17p13.1) and 36% with del(11q22.3). Using NCI 1996 criteria, the OR rate was 31%, and all responses were partial. The objective response rate was 25% for patients with del(17p13.1), 30% for those with del(11q22.3), and 39% for those with bulky lymphadenopathy. The most frequent serious adverse events were infections (32%), tumor lysis syndrome (19%), diarrhea (17%), and febrile neutropenia (15%). Flavopiridol has demonstrated activity in relapsed CLL with high-risk cytogenetics and bulky lymphadenopathy,[119] but further study is needed to establish its ultimate role in treating CLL.

SNS-032

Based on the results with flavopiridol, several CDK inhibitors are undergoing clinical study. The MD Anderson Cancer Center has an ongoing phase I study of SNS-032, a selective inhibitor of CDK 2, 7, and 9, which are involved in cell cycle regulation and regulate RNA polymerase II-dependent transcription. To date, 17 patients with CLL have been treated with total doses of 15 to 100 mg/m^2 infusions. Although no drug-related DLTs were observed with in the cohort receiving 50 mg/m^2, tumor lysis syndrome was noted in all CLL patients treated at a dose of 75 mg/m^2, and 1 patient experienced a DLT of vascular leak syndrome. One patient treated at a dose of 100 mg/m^2 experienced tumor lysis syndrome and a DLT of transaminitis, but no objective responses have been observed.[120]

Dinaciclib

Dinaciclib (SCH 727965) is a selective CDK 1, 2, 5, and 9 inhibitor that appears to have a better therapeutic window than flavopiridol has[121] and is being investigated in a phase I trial in patients with relapsed or refractory CLL. Preliminary results from this study included 8 PRs among 23 evaluable patients. Further study of this agent in CLL is warranted.[122]

Small Modular Immunopharmaceuticals

In CLL, a small modular immunopharmaceutical (SMIP) has been developed to target CD37, an antigen that is involved in the regulation of B-cell function but is not required for B-cell development. CD37 is a heavily glycosylated cell surface protein expressed constitutively at high levels on normal and transformed B cells across a wide range of maturational stages.[123] TRU-016 is a novel humanized anti-CD37 SMIP protein, which is a single-chain Fv-Fc fusion protein representing a structural modification of a CD37 antibody that lacks the C_H1 domain.[123] In vitro studies with the chimeric version of TRU-016 demonstrated that it was a potent inducer of apoptosis and ADCC against CLL cells.[124] In a phase I study in 57 patients with advanced CLL and SLL, TRU-016 was well tolerated.[123] The median number of prior treatments was 4. In this study, 59% of patients were refractory to their prior therapy and 57% had high-risk genomic features. Seven PRs were reported, including 2 PRs in patients with del(17p13.1). The OR rate for patients with 1 or 2 prior therapies was 44% (7 out of 16), all PRs, and the median reduction in peripheral lymphocytes was 92%. No responses were obtained in 41 patients who had 3 or more prior therapies, although a median reduction in lymphocytes of 67% was reported. There were no serious adverse events or DLTs at the highest dose of 20 mg/kg, so a maximum tolerated dose was not reached. Given the demonstrated single-agent clinical activity of TRU-016 and the its synergistic or additive impact with multiple agents in preclinical models, further dose escalation and combination drug trials of TRU-016 have been initiated.

Bcl-2 Inhibitors

Bcl-2, an antiapoptotic protein, is overexpressed in CLL cells and results in resistance to chemotherapy.[125] Antisense oligonucleotides that bind mRNA can target specific proteins and induce enzymatic cleavage of the message, thereby preventing protein translation.[126]

Oblimersen

Oblimersen, an antisense oligonucleotide, downregulates the Bcl-2 protein and potentiates the cytotoxic activity

of commonly used CLL agents including fludarabine, rituximab, and alemtuzumab.[127] Clinically, oblimersen displayed modest single-agent activity in a phase I/II trial in relapsed/refractory CLL.[128] A randomized phase III trial was conducted to compare FC to FC plus oblimersen in 241 patients with relapsed or refractory CLL.[127] Twenty patients (17%) in the FC/oblimersen group and 8 patients (7%) in the FC-only group achieved a CR or a nodular PR. TTP and survival were both prolonged in patients who achieved a CR or a nodular PR ($P < .0001$). A 5-year follow-up of these patients demonstrated a significant survival benefit for those who had fludarabine-sensitive disease or who achieved a CR or a PR with FC plus oblimersen.[129] Adverse effects of oblimersen included nausea, thrombocytopenia, tumor lysis syndrome (rare), and cytokine release reactions (rare). Oblimersen remains an investigational drug.

Gossypol

Gossypol (AT-101), an orally active cottonseed extract derivative, acts as a BH3-mimetic, binding to the BH3 pocket of the Bcl-2 family antiapoptotic proteins (Bcl-2, Bcl_{XL}, Bcl_{W}, and Mcl).[130,131] Gossypol induces apoptosis of CLL cells and overcomes microenvironment-mediated resistance while sparing normal stromal cells.[132] It also enhances the cytotoxicity of rituximab for CLL cells in vitro.[133] Preliminary results of an ongoing phase II study of gossypol combined with rituximab in patients with relapsed or refractory CLL demonstrated a PR in 5 of 12 patients (42%). Patients received a regimen of 30 mg daily for 3 out of every 4 weeks, with rituximab, 375 mg/m^2 for 12 doses (total dose = 4,500 mg/m^2) on days 1, 3, 5, 8, 15, 22, 29, 31, 33, 40, 57, 59, and 61.[134] Intermittent administration of gossypol with a "pulse" dose regimen appeared to be associated with an increased proapoptotic effect in vivo and higher plasma concentrations, as well as reduced toxicity, when compared with daily dosing. The most frequent toxicities were gastrointestinal effects, fatigue, and neutropenia.

Obatoclax

Obatoclax mesylate (GX15-070) is a synthetic small molecule pan–Bcl-2 antagonist with in vitro activity against CLL cells. In a phase I trial, obatoclax was administered to patients with advanced CLL at doses ranging from 3.5 mg/m^2 to 14 mg/m^2 as a 1-hour infusion and from 20 mg/m^2 to 40 mg/m^2 as a 3-hour infusion every 3 weeks.[135] Although the clinical activity of this agent was limited (4% PR; 1 of 26 patients), patients with anemia (3 of 11 patients) or thrombocytopenia (4 of 14 patients) experienced improvements in hemoglobin and platelet counts. Dose-limiting reactions were neurologic (somnolence, euphoria, ataxia) and were associated with the infusion. Further evaluation in less heavily pretreated patients and in combination with other therapeutic agents is warranted.

Navitoclax

Navitoclax (ABT-263), a novel, orally bioavailable, small molecule binds with high affinity to Bcl-2, Bcl_{XL}, and Bcl_{W}, promoting apoptosis. A phase I trial demonstrated that oral navitoclax monotherapy was well tolerated and had antitumor activity in patients with CLL with thrombocytopenia as the DLT.[136] An international phase I dose-escalation study has been initiated to evaluate the safety and pharmacokinetics of oral navitoclax in combination with FCR or with bendamustine/rituximab in patients with relapsed/refractory CLL.[137] Accrual on this trial is ongoing, but preliminary responses in the bendamustine/rituximab arm are encouraging among 4 assessable patients who received 2 cycles: 1 CRi in a patient with del(17p), 2 unconfirmed CRs, and 1 PR in a patient with del(17p). Data are limited in the FCR portion of the study.

SPC2996

SPC2996 is a recently developed specific Bcl-2 mRNA antagonist.[138] Patients with relapsed CLL were treated with a maximum of 6 doses of SPC2996 (0.2 mg/kg to 6 mg/kg) in a multicenter phase I trial.[139] SPC2996 caused a 50% reduction of circulating lymphocytes in 5 of 18 patients (28%), which was found to be independent of its immunostimulatory and anti–Bcl-2 effects. Further studies with this agent are warranted.

Kinase-Targeted Therapy

Unlike chronic myeloid leukemia, where a genetic lesion results in the production of Bcr-Abl tyrosine kinase, CLL does not have a distinct genetic lesion associated with pathophysiology or biology of the disease. However, in recent years, a few kinases have been identified that, in comparison with normal lymphocytes, are overexpressed, constitutively active, or activated by microenvironment factors in pathogenic CLL cells.[140] Selective small-molecule inhibitors of these kinases are currently being tested in early-phase clinical trials in patients with relapsed or refractory CLL, thus opening a new pharmacopoeia for CLL where nucleoside analogs, alkylating agents, and monoclonal antibodies have long been the mainstay of therapy.

CAL-101

In CLL, the PI3K pathway is constitutively activated and dependent on PI3Kδ. CAL-101 is an isoform-selective inhibitor of PI3Kδ that inhibits PI3K signaling and induces apoptosis of CLL cells in vitro. A phase I study of CAL-101 in 37 patients with relapsed or refractory CLL was recently reported.[141] Most patients had refractory disease (65%), bulky lymphadenopathy (81%), and adverse cytogenetics of del(17p), del(11q), or both (63%). The median number of prior therapies was 5. CAL-101 was administered orally once or twice per day continuously in 28-day cycles for up to 12 cycles. Symptomatic adverse events were infrequent and not clearly related

to the study drug. CAL-101 reduced lymphadenopathy in all of the patients, and 91% achieved a lymph node response (≥ 50% reduction in target nodal lesions). A transient increase in peripheral absolute lymphocyte counts of > 50% from baseline was observed in 60% of patients, which suggested drug-mediated lymphocyte redistribution. The OR rate was 33% (all PRs) and the median duration of response had not been reached; 75% of patients with CLL-related thrombocytopenia had either an improvement to > 100,000/μL or a > 50% increase from baseline. These results support further evaluation of CAL-101 alone and in combination with other agents.

Another phase I trial studied CAL-101 in combination with rituximab and/or bendamustine in patients with heavily pretreated relapsed or refractory B-cell indolent NHL and CLL.[142] All patients received CAL-101 100 mg orally twice per day in 28-day cycles for up to 12 cycles. Patients also received rituximab 375 mg/m^2 administered weekly for 8 weeks, starting on day 1 of cycle 1, or bendamustine 90 mg/m^2 administered on days 1 and 2 of each cycle for 6 cycles. Preliminary clinical response assessments available from 3 patients with CLL showed a PR in 2 of 3 patients. Enrollment is ongoing and dose escalation of CAL-101 is planned.

Fostamatinib

B-cell antigen receptor signaling is increasingly recognized as a key factor in promoting clonal expansion in various B-cell malignancies, including CLL. Engagement of the B-cell antigen receptor activates Syk (spleen tyrosine kinase), which leads to a number of downstream events that promote cell survival and growth. Therefore, inhibition of Syk represents a novel therapeutic approach in CLL.[143] A multicenter phase I/II clinical trial of fostamatinib disodium (R788), the first clinically available oral Syk inhibitor, in patients with recurrent B-cell NHL was reported.[144] The 11 patients with SLL/CLL enrolled in the study achieved an OR rate of 55% (all PRs) and a PFS of 6.4 months. Common toxicities included diarrhea, fatigue, cytopenias, hypertension, and nausea. Preclinical studies of other Syk inhibitors such as R406 and two highly selective Syk inhibitors (P142-76 and P505-15) and a multikinase inhibitor (P420-89) demonstrated responses in CLL cells supporting the clinical development of these promising new agents in patients with CLL and other selected B-cell malignancies.[143,145]

PCI-32765

One of the downstream kinases activated by Syk is Bruton tyrosine kinase (BTK), which is an intermediary enzyme in the B-cell antigen receptor signaling pathway. It is a cytoplasmic enzyme that is essential in B-lymphocyte development, survival, and signaling. PCI-32765 is a potent, selective, irreversible, and orally bioavailable small molecule inhibitor of BTK that has preclinical activity in B-cell malignancies.[146] A phase I trial of PCI-32765 was conducted in patients with CLL/SLL who had relapsed or refractory disease after more than one prior treatment regimen.[147] The study included a second phase IB section that included a cohort of patients aged ≥ 65 years with CLL/SLL who required treatment and were "treatment-naive." The drug was well tolerated, with infrequent grade 3 toxicities. Of the 30 patients enrolled in the study, 14 were evaluable for response. The OR rate was 64% (1 CR, 8 PRs, and 4 stable disease). Both studies are ongoing and open to enrollment. A similar response rate (60% OR rate, 6% CR) and safety profile were seen in a separate phase I trial of PCI-32765 in various B-cell malignancies that included 15 patients with CLL.[148]

Dasatinib

Preclinical studies have shown that **CLL** cells overexpress Lyn kinase (an Src-family kinase). In vitro inhibition of Lyn kinase leads to apoptosis of the **CLL** cells. A phase I clinical trial of single-agent dasatinib, a Lyn inhibitor, showed a 20% OR rate and reported myelosuppression as the major toxicity.[149] Another phase II study of dasatinib monotherapy showed a lack of efficacy in heavily pretreated CLL patients, with an OR rate of 6% and a high incidence of neutropenia.[150] Phase I clinical trials of dasatinib in combination with rituximab or lenalidomide are currently ongoing.

Heat Shock Protein 90 Inhibitors

The zeta-associated protein of 70 kDa (ZAP70) is expressed in patients with aggressive CLL. Heat shock protein 90 (Hsp90) is a ubiquitous molecular chaperone that is necessary for the expression and activity of the tyrosine kinase ZAP70, which is expressed aberrantly in 45% of patients with CLL. ZAP70+ CLL cells expressed activated Hsp90 with high binding affinity for Hsp90 inhibitors. Treatment with Hsp90 inhibitors such as 17-AAG and 17-DMAG induced ZAP70 degradation and apoptosis in CLL cells but not in T cells, and it also impaired B-cell receptor signaling in leukemia cells.[151] Hsp inhibitors are currently being investigated in a phase I clinical trial in relapsed CLL (NCT01126502, clinicaltrials.gov).

Histone Deacetylase Inhibitors

Several studies have demonstrated that histone deacetylase (HDAC) inhibitors, including depsipeptide (a potent inhibitor of class I HDAC enzymes), MS-275 (a selective class I HDAC inhibitor), and valproic acid (a nonselective HDAC inhibitor), can alter histone modifications in CLL and lead to selective cytotoxicity of CLL cells.[152-154] In vitro studies have shown that valproic acid restores the apoptotic pathways in CLL cells and thus their ability to undergo apoptosis.[155] A phase I clinical trial of depsipeptide included 10 patients with CLL treated with depsipeptide 13 mg/m^2 given intravenously on days 1, 8, and 15.[154] The most common toxicities were progressive fatigue, nausea, and other constitutional symptoms

that prevented repeated dosing. Several patients had evidence of antitumor activity following treatment, but no PRs or CRs were noted by NCI criteria. Depsipeptide effectively inhibited HDAC in vivo in patients with CLL, but this schedule of administration was limited by progressive constitutional symptoms. Future studies with depsipeptide should examine alternative administration schedules. Another phase I trial studied belinostat, a novel HDAC inhibitor, in patients with advanced hematologic malignancies refractory to standard therapy or for whom no standard therapy exists.[156] No responses were noted in these heavily pretreated patients. However, disease stabilization was observed in several patients including the 2 patients with CLL enrolled in the study. MGCD0103 is an orally available, aminophenylbenzamide small-molecule HDAC inhibitor that selectively targets class I (HDAC isotypes 1, 2, and 3) and class IV (HDAC isotype 11) enzymes. A phase II clinical trial was performed, starting at a dose of 85 mg/day and administered 3 times per week.[157] Twenty-one patients with relapsed or refractory CLL received a median of 2 cycles of MGCD0103 (range, 0–12). All patients had previously received fludarabine, 33% were fludarabine-refractory, and 71% had del(11q22.3) or del(17p13.1). No responses according to NCI 1996 criteria were observed. Grade 3/4 toxicity consisted of infections, thrombocytopenia, anemia, diarrhea, and fatigue. Future investigations in CLL should focus on broad HDAC inhibition, combination strategies, and approaches to diminish constitutional symptoms associated with this class of drugs. Several phase I and II studies are currently ongoing with various HDAC inhibitors as a single agent or in combination with other agents in CLL.

Conclusions

Treatment strategies in CLL, as a disease of the elderly, should remain highly individualized. The watch-and-wait approach remains standard for asymptomatic patients. Patients who relapse after a disease-free period of over 1 year (or 2 years following chemoimmunotherapy) are considered to be treatment-sensitive and may receive the same regimen used for initial treatment.[158] Those who relapse within 6 months of completing an initial fludarabine-containing regimen or who progress during therapy are considered fludarabine-refractory.[159] Additionally, since more effective regimens tend to be more toxic, it is important to assess the health status of the patient and define the goals of therapy before making treatment decisions. The goal of therapy in older patients with multiple comorbidities may focus on palliation of symptoms, while in fit or younger patients who can tolerate more intense regimens, the goal of therapy may be to maximize their likelihood of long-term survival, with the ultimate goal in high-risk patients of providing allogeneic stem cell transplantation. Options for refractory patients include alemtuzumab with or without rituximab (although not for bulky lymphadenopathy), bendamustine with or without rituximab, and high-dose corticosteroids with rituximab, ofatumumab, and combination regimens. Alemtuzumab and high-dose corticosteroids are associated with significant toxicity and prolonged immunosuppression. Since CLL itself causes immune dysregulation, serious and life-threatening infections often occur after utilizing immunosuppressive agents. Guidelines for the use of alemtuzumab include a mandatory weekly standardized, qualitative polymerase chain reaction assay to test for reactivation of CMV, cotrimoxazole prophylaxis to prevent *Pneumocystis jirovecii* infection, and acyclovir prophylaxis to protect against herpes viruses.[160] If CMV infection is noted, pre-emptive therapy should be initiated with ganciclovir or foscarnet, and treatment with alemtuzumab should be interrupted.[160] Finally, the activity of novel targeted agents, although still in early stages of clinical investigation, has shown potential to change the future treatment of CLL, for which allogeneic SCT remains the only potentially curative therapy. Continued advances in the research of CLL and enrollment of patients in clinical trials will aid in the pursuit of a future cure for CLL.

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